REGENERATION IN THE SPINAL CORD

OF *TRITURUS VIRIDESCENS*

A THESIS

SUBMITTED TO THE FACULTY OF ATLANTA UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR

THE DEGREE OF MASTER OF SCIENCE

BY

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DEPARTMENT OF BIOLOGY

ATLANTA, GEORGIA

AUGUST 1958

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ACKNOWLEDGMENT

I wish to express my gratitude to my adviser, Dr. M. L. Reddick, and to Mr. W. B. LeFlore for their constructive criticisms during the course of this research.

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# CHAPTER I

## INTRODUCTION

It is generally accepted that regeneration occurs in the central nervous system of fish and amphibia after transection. Tuge and Hanzawa ('37) transected the spinal cords of adult teleosts and demonstrated that functional and morphological restoration of the cord took place two and one half months after operation, if connective tissue did not prevent, or if the space between the cut ends of the cord was not too great.

Hooker ('15) made a study of the spinal cords of tadpoles of *Rana sylvatica* and *Rana catesbiana* during the tail bud and hind limb stages in order to analyze the processes leading to its reunion after complete transection. Hooker's study revealed that reunion of the transected cord is accomplished by: (a) the development of nerve fibers from the motor cells of each segment of the cord, (b) the growth of sensory axons from the cut surface of the posterior stump, and (c) the elongation of both ends of the transected cord toward each other by proliferation of the ependymal cells of the central canal. He concluded that under favorable conditions, the spinal cords of frog embryos would regenerate after being completely severed in the cervical region.

The purpose of this investigation was to determine the regenerative capacity of the cervical region of the spinal cord of adult *Triturus viridescens* following its transection.

## CHAPTER II

## REVIEW OF LITERATURE

Tuge and Hanzawa ('37) sectioned the cord of adult teleost to determine the regenerative capacities of the spinal cord. Japanese rice minnows, after section of the cord, were kept alive for varying periods of time. They were killed at intervals, over a period of three months, after transection of the cord. After decalcification, pieces were cut sagittally at 8 u. into serial sections and stained with Delafield's hematoxylin and eosin. Histological studies revealed that if connective tissue did not prevent, or if the space between the cut ends of the cord was not too great, morphological regeneration took place. Characteristics of the regenerated cord which differed from the normal cord were: irregular distribution of nerve fibers; irregular course of the central canal, and smaller diameter of the cord.

Holtzer ('51) performed a series of experiments on the spinal cords of embryonic stages of *Amblystoma punctatum*, *Amblystoma opacum* and *Triturus torosus* in order to determine to what extent the cord would regenerate. Unilateral extirpation of segments three, 4 and 5 was accomplished by a paramedian incision extending from segment two to segment 6. All operations were performed on the right side of the neural tube and the left side was left undamaged. At intervals after the operation serial sections of the cord were cut sagittally and the Bodian method was used to stain them. Holtzer's study revealed that restitution was achieved by mobilization of the cells across the midline from the intact left half to the operated right half of the cord.

Hooker ('15) transected the cords of tadpoles of *Rana sylvatica* and *Rana catesbiana* during the tail bud and hind limb stages. The operation consisted of complete transection of the spinal cord and notochord in the cervical region with Noyes iridectomy scissors. Serial sections of the cord were cut sagittally and stained with: Mallory's connective tissue stain; iron hematoxylin and congo red, and Ehrlich's hematoxylin. Hooker's results showed, that under favorable conditions, the spinal cords of frog embryos would regenerate after being completely severed in the cervical region during the tail bud and hind limb stages. This regeneration was accomplished by: (a) the development of nerve fibers from the motor cells of each segment of the cord; (b) the growth of sensory axons from the cut surface of the posterior stump, and (c) the elongation of both ends of the transected cord toward each other by proliferation of the ependymal cells of the central canal. The elements which entered into the regenerated part of the cord were derived entirely from the original cord. According to Hooker, complete reunion of the cord may be prevented by the interposition of mechanical obstacles to regeneration, or by too great a separation of the cut ends of the cord from each other.

Hooker ('25) performed a series of experiments similar to the experiments described above, to determine the processes involved in the restoration of anatomical continuity in the completely transected cord of <u>Rana sylvatica</u> tadpoles operated on during the tail bud and hind limb stages. The spinal cord was completely transected in the cervical region with a pair of Noyes iridectomy scissors. Serial sections of the cord were made in sagittal and transverse planes at intervals during a three month period after the operation. The sections were stained with Mallory's connective tissue stain and iron hematoxylin and congo red. This study revealed that the gross form and normal structure of the spinal cord of frog tadpoles may be restored, following its transection, at any embryonic stage. Restoration of anatomical continuity of the cord may be restored by: (a) the outgrowth from both ends of the transected cord of nerve fibers from neurons situated in the original cord; (b) the proliferation of the ependymal cells of the central canal of the old cord until the central canal of the new cord is re-established, and (c) the proliferation of indifferent cells in the original cord. These indifferent cells migrate and differentiate into neuroblasts and spongioblasts which assist in the restoration of the structure the new cord. Hooker concluded that complete restoration of normal function usually occurred after there was complete regeneration of the cord.

Clemente and Windle ('54) transected the spinal cord of the adult cat to determine its regenerative capacity. Control and experimental groups of animals were subjected to the same type of operation; however the experimental animals were administered injections of piromen or adrenal cortical trophic hormone whereas the control group was not subjected to these substances. The animals were anesthetized with nembutal and the operations were performed aseptically. After a laminectomy involving the vertebral arch, the spinal cord was transected with a Bard Parker scalpel. The cats were killed at intervals over a period of 19 months, after transection of the cord. The transected area and several segments of the cord rostral and caudal to the lesion were then dissected out and placed in a fixing solution for hardening. The cord was cut sagittally at 10 u. into serial sections and the Ransom pyridine technique was used for staining the sections. This study revealed that the regenerating fibers of the cord of the control group to which no drugs had been administered encountered the piaglial membrane,and could not pass through this barrier. The regenerating cord of the experimental animals did not encounter the piaglial membrane, however, even in cases where the membrane was not present at the wound site, very little morphological reconstitution of the cord took place.

Clearwaters ('54) transected the cords of chick embryos after 40 to 120 hours of incubation, in order to determine the regenerative capacity of the spinal cord. The cords of chicks several days after hatching were also transected for study. During various periods of incubation, a window one centimeter in diameter was cut in the shell over the chick embryos. The cords of the chick embryos were transected with a 60 cycle vibrating knife just caudal to the wing buds at the level of the 21st or 23rd somite. In the hatched chicks, a hole was made in the vertebral column of the thoracic region and a dissecting needle was used to section the cord. Animals in both groups, two to 6 days after operation, were sacrificed for histological study. Sagittal and transverse serial sections of the cords were cut at 10 u. In younger specimens the entire embryo was sectioned serially, but the spinal column of older chicks was dissected out and the bone was decalcified. The sections were stained with the pyridine silver technique. Clearwaters' study revealed that the embryonic chicks showed complete repair by the 6th day after the operation, but the normal "architecture" of the cord was not restored. Chicks operated on after they had been hatched showed indication of nerve fiber regeneration by the 5th week after operation, but nerve fibers did not cross the transected area and functional recovery did not occur.

# CHAPTER III

## MATERIALS AND METHODS

The experimental animals used for this investigation were adult *Triturus viridescens* from 8 to 10 cm. in length. The stock animals were kept in a rectangular aquarium which contained an ample supply of vegetation to make the laboratory conditions as similar to the normal habitat as possible. The *Triturus* were not fed in the stock aquarium because of danger from fungus infection. They were removed from the aquarium, placed in battery jars and fed. After they were fed each specimen was rinsed in tap water and placed again in the aquarium. Anesthesia was not used during the pre-operative phase of the experiment. The animals were placed in beakers of water which were then placed in a refrigeration compartment. The specimen was allowed to remain in the refrigeration compartment until its motility was slowed considerably. At this point the animals were ready for operation.

The operation consisted of a complete transection of the spinal cord in the cervical region between spinal segments one and 4. Transections were performed under aseptic conditions and both the vertebral column and spinal cord were cut with the same stroke of the blade. The cut ends of the cord could in all instances be seen under the stereoscopic microscope following the operation.

Post-operative animals were placed in battery jars which contained tap water after the battery jars had been rinsed in a 5% solution of potassium permanganate. They were allowed to remain in the battery jars until they were killed. Animals which had been operated on were sacrificed at intervals of one week for 10 weeks. After each specimen was killed an entire area of the vertebral column, including the spinal cord was removed. Both ends of the area removed were cut beyond the scar tissue in order to make sure that some of the old cord was present. The tissue was fixed in Bouin's, decalcified, cut into sagittal serial sections 10 u. thick and stained according to the Bodian method. Some sections were stained with iron hematoxylin.

# CHAPTER IV

## EXPERIMENTAL RESULTS

The experimental data compiled from this investigation gives an account of regeneration in the spinal cord of adult Triturus viridescens which took place over a period of 10 weeks. Except in cases where fungus caused infection, healing of the wound was normal. The spinal cords of 24 animals were transected, 5 of the animals died as a result of fungus infection or from excessive bleeding following the operation.

### A Description of the Normal Cord

The central canal can be seen passing in a straight line through the gray matter of the spinal cord. Ependymal cells can be seen lining the inner surfaces of the central canal and neuroglial cells can be seen surrounding the outer surface. The gray matter of the cord is composed of numerous nerve cells including their dendrites and axons. Motor nerve cells can be distinguished because they are larger than the nerve cells of the association neurons and neuroglia which are also found in the gray matter. The white matter of the cord can be seen on the outer surfaces of the gray matter to consist of longitudinally coursing nerve fibers supported by neuroglia. The nerve fibers of the white matter display continuity and noticeable breaks in their continuity or deviation from their course cannot be seen in the normal cord (fig. 1).

### A Description of the Regenerated Cord

One week regenerates.--The nerve cells of the gray matter and the fibers of the white matter have closed the gap between the cut ends of the cord. However, the nerve cells of the gray matter and the fibers of the white matter in the transected area appear indifferent in character, The cells are not as granular as those of the normal cord and the operated cord shows irregular distribution of nerve cells. Even at this early stage, a considerable degree of morphological reconstitution was observed. The site of transection is evident because scar tissue can be seen adjacent to the transected area of the cord (fig. 2).

Two week regenerates.--The nerve fibers, and nerve cells of the gray matter are not as thickly populated in the transected area of the cord. The meninges have already healed. Morphological reconstitution can definitely be seen. Scar tissue adjacent to the site of the transected cord is evident (fig. 3).

Three week regenerates.--Bone of the vertebral column can be seen close to the transected area of the cord. The diameter of the cord is smaller in the transected area as compared with portions anterior and posterior to it. The nerve cells of the gray matter are more scarce than in the normal cord. The central canal is distorted (fig. 4).

Four week regenerates.--The central canal shows irregularity in the transected area, otherwise morphological restoration of the transected cord appears normal. Scar tissue of the epidermis and musculature surrounding the vertebral column is still present (fig. 5).

Five week regenerates.--The regenerated area of the cord appears normal except for more scarcely populated fibers of the white matter. The site of the transection could be identified by scar tissue adjacent to it (fig. 6).

Six week regenerates.--The cord shows complete restoration of the transected area. The gray matter and the fiber tracts of the white matter appear normal. The only indication that the cord had been transected is the scar tissue contiguous to the transected cord (fig. 7).

Seven week regenerates.--The cord shows a high degree of morphological restoration in the transected area, however, the regenerated area is slightly smaller in diameter than the cord anterior and posterior to it. Fibers of the white matter appear distorted (fig. 8).

Eight to ten week regenerates.-- The operated cords show complete morphological restoration. The nerve cells are normally distributed and the fibers of the white matter show normal confluence (figs. 9 and 10).

## CHAPTER V

## DISCUSSION

Results from this investigation show that the spinal cord of adult Triturus viridescens is capable of extensive morphological and histological regeneration after the cord is transected. One important factor which seems to have bearing on the extent and rate of regeneration is the amount of space left between the cut ends of the cord following complete transection. Whereas some investigators removed an entire segment of the spinal cord including the vertebral column, the spinal cord in this experiment was transected without removal of an entire segment. This may have contributed to the rate of, and extensive morphological regeneration which took place in the transected cord.

The reunion of the cord is accomplished by nerve fibers which bridge the gap between the cut surfaces of the cord to re-establish anatomical continuity. These fibers were derived entirely from the area of the cord anterior and posterior to the site of transection. There was no indication that sources, other than the cord, supplied material for the reconstitution of it. This observation is in accord with one of those made by Hooker ('25). He found that one means by which transected cords of frog tadpoles were re-established was by the growth of axons from neurons situated in the original cord.

It was observed that the operated cord of Triturus often showed an irregular distribution of the nerve cells of the gray matter, and an irregular course of the central canal. Some morphological reconstitution was always evident, but the operated cord had a smaller diameter than the normal cord in some instances. These observations are in agreement with the results of Tuge and Hanzawa ('37) who studied the regenerative capacity of the adult teleost. According to them: the regenerated cord was smaller in diameter; the central canal was irregular in course, and the distribution of the fibers was abnormal.

## CHAPTER VI

## SUMMARY AND CONCLUSIONS

1. The cervical cord region of adult _Triturus viridescens_ was transected and histological and morphological observations were made on its regeneration for from one to 10 weeks.
2. The spinal cord of adult _Triturus viridescens_ is capable of morphological and histological reconstitution if the space between the cut ends of the cord is negligible following transection.
3. In most cases the operated cord seldom reached normal proportions, and the central canal was irregular.
4. Regeneration was complete 6 to 8 weeks after the cord was transected.

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PLATE I*

(Explanation of Figures)

*All figures are photomicrographs of stained sections (X100).

(Explanation of Figures)

1. Sagittal section through the unoperated cord of _Triturus viridescens_ showing the central canal passing in a straight line through the gray matter; motor and neuroglial cells of the gray matter, and longitudinally coursing fibers of the white matter.

2. Sagittal section through the transected cord of _Triturus_ one week after operation showing nerve fibers which have closed the gap between the cut ends of the cord, and nerve cells of indifferent character.

3. Sagittal section through the transected cord of _Triturus_ two weeks after operation showing more sparsely populated nerve fibers and the healed meninges.

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PLATE II*

(Explanation of Figures)

*All figures are photomicrographs of stained sections (X100).

(Explanation of Figures)

4. Sagittal section through the transected cord of *Triturus* three weeks after operation showing bone of the vertebral column close to the transected area of the cord, and smaller diameter of the cord.

5. Sagittal section through the transected cord of *Triturus* 4 weeks after operation showing irregularity in the transected area.

6. Sagittal section through the transected cord of *Triturus* 5 weeks after operation showing regenerated area which appears normal with the exception of more sparsely populated fibers of the white matter.

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PLATE III*

(Explanation of Figures)

*All figures are photomicrographs of stained sections (X100).

(Explanation of Figures)

7. Sagittal section through the transected cord of Triturus 6 weeks after operation showing complete restoration morphologically.

8. Sagittal section through the transected cord of Triturus 7 weeks after operation showing high degree of morphological restoration but slightly smaller cord diameter than normal.

9. Sagittal section through the transected cord of Triturus showing complete morphological and histological restoration 8 weeks following operation.

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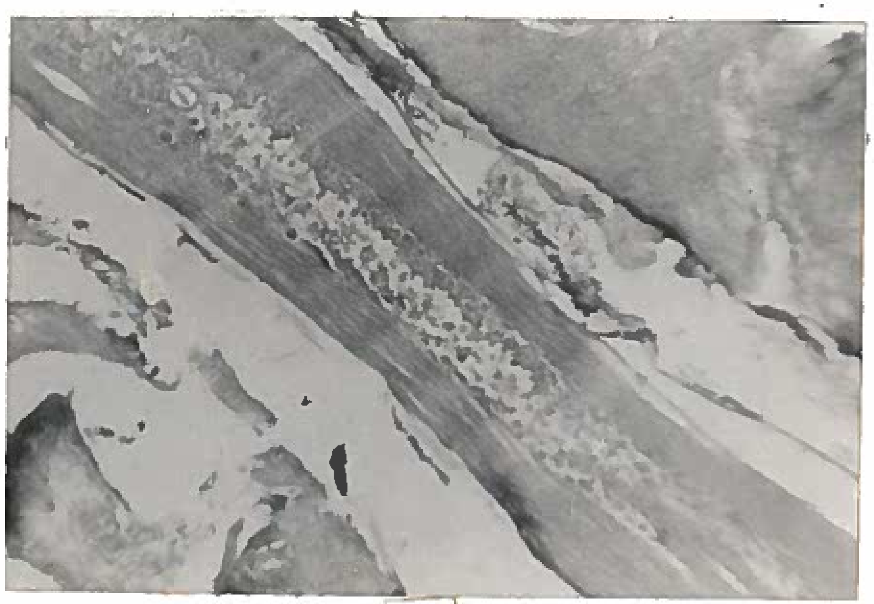

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PLATE IV*

(Explanation of Figures)

*All figures are photomicrographs of stained sections (X100).

(Explanation of Figures)

10. Sagittal section through the cord of Triturus showing complete morphological restoration 10 weeks after transection.

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